Docket No. HER-0071
Serial No. 10/530,539
Patent Application

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Sajjadi, Fereydoun Ghotb
Art Unit	:	1633
Applicants	:	Ono, Esturo and Uede, Toshimitsu
Filed	:	September 19, 2005
For	:	Method for producing a mammal provided with resistance to an alpha-herpes virus mediated infection and mammal obtained by implementing said method and said mammal's progeny.

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION (UNDER 37 CFR §1.132) OF DR. PIERRE CHEREL

Sir:

Dr. Pierre Cherel hereby declares:

My Curriculum Vitae is attached herewith;

THAT, I am a veterinary surgeon and the Director of the Research & Development Department at the company France Hybrides;

THAT I have directly or indirectly conducted experiments with transgenic pigs that were produced by the methods described in the U.S. Patent Application mentioned above (hereinafter, "Ono application");

THAT I have reviewed the Office Action dated September 5, 2008, and the references cited thercin;

AND, being thus duly qualified as an expert in the field of the invention, I do hereby declare:

Provided below are additional data obtained using the teachings of the subject application. Before introducing that data, and in addition to the Remarks included with the concurrently submitted Response, I would like to first comment on statements in the office action alleging variable outcomes from microinjection procedures. Any perceived issues regarding such variability, generated by position effects resulting from random insertion of the transgene, did not affect the overall efficiency of the subject methods. The subject methods were used to generate both murine and porcine transgenic lines made resistant to *in vivo* viral challenges. The presently claimed methods were thus shown to be repeatable, effective, and efficient in generating expected outcomes, i.e. lines of resistant mice or pigs.

Specifically regarding the cited Fiume (U.S. Patent No. 6,469,155), I would also like to add that Fiume relates to a receptor identification process, which stands alone as a generic tool. Fiume involved making resistant cell lines (not whole animals) by a described selection process, and testing expression of a library of proteins in those cells, to infer a receptor role to proteins showing restored sensitivity to viral infection. This was done with the aim of viral receptors identification, as an essential primer for antiviral therapeutic targets definition.

If Fiume mentions some "cells made resistant," that might be true only with respect to that "receptor discovery tool." Fiume does not teach or suggest a transgene / transgenic mammal approach for doing so, as taught by the subject application. Even where soluble proteins are considered by Fiume for their virus binding properties as such, it is only in the context of a medicament discovery, and never within the context of a transgenic rodent (which would have no value as a model for therapeutics testing).

In addition, the soluble fusion proteins of Fiume are used by Fiume to define targets for antibodies. One main difference between the soluble proteins of the subject application and Fiume is that Fiume relates to drugs / drug discovery, while the subject application shows that

transgenic mammals expressing the specified fusion genes are resistant because of the fusion protein production. This was in no way taught, suggested, or predictable in light of Fiume.

We obtained the following experimental data from transgenic pigs expressing fusion proteins of the subject application, and using that methodology. This data further establishes that the pigs expressing the fusion protein of the subject application are resistant to infection by the PRV. Experimental design and the results of the experiments will now be described.

Experimental design:

Two lines of the transgenic pigs were generated by micro injection of SalI-SnaB1 fragment from construct described in Figure 6 (pVCC HveC-hFc) and Sequence 2 of subject patent application. F2 piglets where produced as progeny of transgenic F1 hemizygous boars and non transgenic sows, with an expected 50/50 segregation rate of transgenic and non transgenic animals. Transgene inheritance and expression where assessed for all F2 piglets by PCR, Southernblot (Transgene inheritance) and ELISA (fusion protein expression in serum), allowing to identify transgenic F2 piglets and control littermates (selected from the same litters but having not inherited transgene, and thus not expressing fusion protein). F2 transgenic piglets and control littermates were raised in the same conditions and subjected to the same lethal PRV challenge, at the age of 20 days. Lines A and B animals in Table I are referring to F2 (2^{nd} generation) piglets from respectively A and B F0 founders (produced by microinjection).

Briefly, the piglets were weaned on day 16^{th} after birth and evaluated for weight gain between day 17 and 19. Proper weight gain between these two days indicated good weaning quality. The piglets were infected on day 20 with 1.5 ml in each nostril, and 1 ml per os of viral inoculum (10^4 plaque forming units PRV/ml NIA strain) and the animals were subsequently observed for weight gain and mortality.

Results:

	Line A		Line B	
	Transgenic	Control littermates	Transgenic	Control littermates
Days 0	11	11	12	12
20 Days post infection	10	0	6	0
Mortality (%)	10	100	50	100

Table I: Table 1 shows the number of piglets surviving 20 day post-infection.

As seen from Table I, piglets from the transgenic lines A and B exhibit only 10% and 50% mortality respectively. Piglets from the control group however, show 100% mortality. Transgenic piglets exhibit significant resistant to PRV infection and substantially reduced lethality compared to the control piglets.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or of any patent issuing thereon.

Further, declarant sayeth not.

March 4th, 2009
Dated

Pierre Cherel
DR. PIERRE CHEREL

FRANCE HYBRIDES Technical Manager resume

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EDUCATION

- DVM in Ecole Vétérinaire d'Alfort (Maisons Alfort, France), 1988

- Trainee research project on Viral Heamorragic Septicemia viral protein and nucleic acid detection in healthy carriers (rainbow trout). 6 months (INRA Jouy, AFSSA) 1989

- DVM Thesis on chloramphenicol pharmacocinétics in fish (rainbow trout) using biocarriers (A.salina) and HPLC. (ENVA, 1990)

- Quantitative genetics training (Mixed model course, Robin Thomson, UK), 1993

- Molecular biology course, (U. of St Louis, Minnesota, USA), 2000

PROFESSIONNAL

Since 1991, work in FRANCE HYBRIDES, french private company specialized in pig breeding, work as a veterinarian on biosecurity and health control programs

Since 1993, technical manager for FRANCE HYBRIDES.

Practical management of the pig breeding program, including estimation of genetic parameters for 5 lines, breeding goal formulation and economic weighting, diversity management, software development.

Reproduction research program development (incl. pig embryo freezing and transfer)
Since 1996, molecular biology lab development: DNA banking, PCR RFLP genotyping
Since 2000, microsatellite genotyping lab set up
Since 2002, high throughput microsatellite genotyping platform setup using robotic PCR setup and 384 wells plates.

From 2000, setup and management of research project focused on detection of QTL and differentially expressed genes associated to loin tenderness and ham colour.